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PRE-APPEAL BRIEF REQUEST FOR REVIEW

Docket Number (Optional): 31175413-002002

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on September 26, 2007

Signature /Michael D Berger #52616/

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Application Number	Filed
10/699,511	October 31, 2003

First Named Inventor: George N. Bennett

Art Unit	Examiner
1637	Calamita, H.

Applicant requests review of the final rejection in the above-identified application. No amendments are being filed with this request.

This request is being filed with a notice of appeal.

The review is requested for the reason(s) stated on the attached sheet(s).
Note: No more than five (5) pages are provided.

I am the

☐ applicant/inventor.

☐ assignee of record of the entire interest
See 37 CFR 3.71. Statement under 37 CFR 3.73 (b) is enclosed.
(Form PTO/SB/96)

☒ attorney or agent of record.
Registration number 52,616

☐ attorney or agent acting under 37 CFR 1.34

Registration number if acting under 37 CFR 1.34 _____

/Michael D. Berger/
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Telephone number

September 26, 2007
Date

NOTE: Signatures of all the investors or assignee of record of the entire interest or their representative(s) are required.
Submit multiple forms if more than one signature is required, see below*.

☒ *Total of 1 forms are submitted

This collection of information is required by 35 U.S.C. 132. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11, 1.14 and 41.6. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Mail Stop AF, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

PRE-APPEAL BRIEF REQUEST FOR REVIEW

Dear Sirs:

Claims 1-7 are pending in the current patent application. All pending claims stand rejected under 35 U.S.C. §103 based on Watson (Biotechniques, 1997) and Ellidge (US5851808), in view of Stahl (Biotechniques, 1993). The claims were first rejected in the Office Action mailed February 22, 2007. After the Applicants' response, the claims were finally rejected on July 26, 2007. Thus, the claims have been twice rejected and are ripe for appeal. Applicants request reconsideration of the final rejection of claims 1-7 in this Pre-Appeal Brief Request for Review for the reasons below.

COMMENTS REGARDING THE CLAIMED INVENTION

Claims 1-7 relate to assembly of PCR fragments on a solid support and removal of those fragments to generate a functional circularized DNA molecule. Specifically claim 1 recites 7 steps (a-g): a) amplifying a first PCR fragment wherein said first PCR fragment comprises **a first site specific recombinase site**; b) generate a 3' overhang and immobilize on solid support; c) amplify another PCR fragment; d) generate a 3' overhang; e) anneal and ligate; f) optionally repeat steps c, d, and e wherein the last PCR fragment comprises a **second site specific recombinase site**; g) **removing and circularizing** the assembled DNA fragment from the solid support with a site specific recombinase.

MISSING ELEMENT NOT FOUND IN THE CITED ART

None of the cited art shows "**simultaneously removing and circularizing**" assembled PCR fragments from a solid support with a recombinase. In the absence of these missing elements, the obviousness rejection cannot be maintained.

35 U.S.C. §103 NON-OBVIOUS SUBJECT MATTER

Obviousness depends on (1) the scope and content of the prior art; (2) the differences between the claimed invention and the prior art; (3) the level of ordinary skill in the art; and (4) any relevant secondary considerations, including commercial success, long felt but unsolved needs, and failure of others. *Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966). (1) The scope and content of the prior art references describe assembly of PCR fragments (Watson), a recombinase system for exchanging expression cassettes *in vitro* (Ellidge), and assembling synthetic oligonucleotides on a solid support (Stahl), see also Examiner's arguments February 22, 2007 and July 26, 2007, pages 2-4. (2) The

differences between the claimed invention and the prior art are **NONE** of the references show "**removing and circularizing** the assembled DNA fragment from the solid support with a site specific recombinase." See Applicant's Response May 17, 2007, page 4-6.

Table 1: Differences between the claimed invention and the prior art			
Claim 1	**Watson**	**Ellidge**	**Stahl**
a) making a first PCR fragment with first and second primers, wherein the second primer comprises a modified nucleotide that can be removed by a DNA repair enzyme, resulting in a 3' overhang, and wherein the first PCR fragment comprises **a first site specific recombinase site**;	NO recombinase site	YES	NO
b) treating the first PCR fragment with a DNA repair enzyme to generate a 3' overhang and immobilizing the first PCR fragment on a solid support or vice versa;	NO Solid Support	NO Solid Support	YES, but no PCR fragments
c) making a second PCR fragment with third and fourth primers, wherein the third and fourth primers each comprises a modified nucleotide that can be removed by a DNA repair enzyme resulting in a 3' overhang;	YES	NO	NO
d) treating the second PCR fragment with a DNA repair enzyme to generate a 3' overhang;	YES	NO	NO
e) annealing and ligating the first and second PCR fragments;	YES	NO	NO
f) optionally repeating steps c, d and e until a last PCR fragment is added to the growing chain to produce an assembled fragment, wherein the last PCR fragment comprises **a second site specific recombinase site**;	NO recombinase site	NO	NO
g) **removing and circularizing** the assembled fragment from the solid support with a site specific recombinase.	Not present	Not present	Not present

The level of ordinary skill in the art (3) is high, a molecular biologist with an advanced degree, but none of the prior references including Watson, Ellidge, or Stahl used a site specific recombinase with a solid support. (4) Relevant secondary considerations of non-obviousness are provided in a Declaration by Dr Bennett (May 17, 2007):

> First, it was unknown if synthetic DNA substrates are active substrates.
>
> Second, DNA topology on a solid support affects recombinase activity.
>
> Finally, enzyme activity is altered in microenvironments on a solid support.

Therefore it was not obvious a recombinase would **remove and circularize** an assembled DNA fragment from a solid support.

COMPETENT DECLARATORY EVIDENCE ESTABLISHES NO REASONABLE EXPECTATION OF SUCCESS

The Declaration of Dr. George N. Bennett, previously submitted, identifies several reasons why one of ordinary skill in the art would not have thought recombination on a solid support predictable or obvious. One important reason relates to the topology of the DNA attached to the solid support:

> **Prior to the present invention, the ability of the CRE protein to function on immobilized DNA was unknown. The use of immobilized DNA for the *Cre/lox* recombination was not thought possible because immobilized DNA has a different topological structure than either native DNA *in vivo* or purified DNA *in vitro*. The *Cre/lox* reaction changes the topological structure of the DNA substrate. A DNA structure tethered to a solid support might not undergo the conformational changes required for recombination. One of ordinary skill in the art would have thought *Cre/lox* recombination was inhibited or impossible on a solid support.**

DNA topology is clearly affected by binding to a solid support (as well as by its linear versus various circular forms), which in turn affects recombinase activity. This is well known in the art.i Thus, even if there was a suggestion in the art to modify the prior art by conducting the reactions on a solid support (and there is none because Stahl only performs ligase reactions on a solid support), there is no reasonable expectation of success where **topology is known to be critical to recombinase function**. A recombinase is **not** a ligase—the assembly of DNA on solid support using ligase per Stahl is **not** analogous and cannot be used to extrapolate success for this unique application of recombinase.

EXAMINER MUST CONSIDER SECONDARY INDICES OF NON-OBVIOUSNESS

According to *In Re John B. Sullivan, et al.* (Fed. Cir. 2007) the Examiner must consider rebuttal evidence provided. "[W]hen a prima facie case is made, the burden shifts to the applicant to come forward with evidence and/or argument supporting patentability." *In re Glaug*, 283 F.3d 1335, 1338 (Fed. Cir. 2002). In this case, Applicants have provided competent evidence of non-obviousness, thus **it is not obvious** a recombinase would be expected to efficiently remove and circularize DNA substrate from a solid support. "The claimed composition cannot be held to have been obvious if competent evidence rebuts the prima facie case of obviousness." *In Re John B. Sullivan, et al.* (Fed. Cir. 2007). **Applicants have demonstrated non-obviousness** therefore the Examiner cannot maintain the obviousness rejection.

THE EXAMINER HAS PROVIDED NO DOCUMENTARY EVIDENCE

Applicants have requested the Examiner substantiate any assertions that a recombinase can reasonably be expected to function on a solid support. However, the Examiner has failed to provide any declaratory evidence, instead, making only conclusory statements that Applicants declaratory

evidence is "unpersuasive," and making unsubstantiated claims about what might be reasonably expected by a recombinase.

Examiner's personal knowledge or mere argument is not competent evidence and is not sufficient to rebut Applicants evidence. Fiers v. Revel, 984 F.2d 1164 (Fed. Cir. 1993) (holding that "the Board did not err in determining that Fiers presented no convincing evidence" where applicant only showed "argument ... 'unsupported by competent evidence, entitled to little or no weight and ... unpersuasive in any event.'"); In re Juillard, 476 F.2d 1380 (C.C.P.A.) ("arguments cannot take the place of evidence").

Examiner is again respectfully requested to fully articulate the rationale in **proper evidentiary form** (e.g., a declaration) according to MPEP 2144.03, so that Applicants may properly rebut same.

CONCLUSION

None of the cited art shows "simultaneously removing and circularizing" assembled PCR fragments from a solid support with a recombinase. The missing elements cannot be pulled from thin air, but must be properly substantiated. In the absence of competent evidence supplying the missing elements (and with competent evidence showing non-obviousness because there is no reasonable expectation of success), the claims have not been shown to be obvious and are in condition for allowance.

Applicants believe that the claims are in condition for allowance and respectfully request that the Examiners grant such an action. If any questions or issues remain in the resolution of which the Examiners feel will be advanced by a conference with the Applicants' representative, the Examiners are invited to contact the attorney at the number noted below. The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Deposit Account No. 50-3420, reference 31175413-002002 (Berger).

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Respectfully Submitted

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[i] There are many papers indicating that DNA topology is critical in the function and outcome of recombinase reactions. A few papers are highlighted herein for convenience, but this feature of recombinase activity is well known in the art:

Kilbride, *et al.*, Determinants of product topology in a hybrid Cre-Tn3 resolvase site-specific recombination system, J Mol Biol. 355(2):185-95 (2006) ("**Many natural DNA site-specific recombination systems achieve directionality and/or selectivity by making recombinants with a specific DNA topology. This property requires that the DNA architecture of the synapse and the mechanism of strand exchange are both under strict control.**").

Vetcher, *et al.*, DNA topology and geometry in Flp and Cre recombination, J Mol Biol. 2006 Apr 7;357(4):1089-104 (2006) ("**Recombination reactions involve particular geometric and topological relationships between DNA target sites at synapsis**, ...").

Grainge, *et al.*, Symmetric DNA sites are functionally asymmetric within Flp and Cre site-specific DNA recombination synapses, J Mol Biol. 320(3):515-27 (2002) ("**Flp and Cre-mediated recombination on symmetrized FRT and loxP sites, respectively, in circular plasmid substrates yield both DNA inversion and deletion. ...** Furthermore, **the observed recombination bias favoring deletion over inversion in a nicked circular substrate** containing two symmetrized FRT sites is consistent with the predictions from Monte Carlo simulations based on antiparallel synapsis of the DNA")

Crisona, *et al.*, The topological mechanism of phage lambda integrase, J Mol Biol. 18;289(4):747- 75 (1999) ("**Bacteriophage lambda integrase (Int) is a versatile site-specific recombinase. In concert with other proteins, it mediates phage integration into and excision out of the bacterial chromosome. Int recombines intramolecular sites in inverse or direct orientation or sites on separate DNA molecules. This wide spectrum of Int-mediated reactions has, however, hindered our understanding of the topology of Int recombination. ... The generality of our results is indicated by our finding that two other members of the integrase superfamily of recombinases, Flp of yeast and Cre of phage P1, show the same intrinsic chirality as lambda Int.**").

Kilbride, *et al.*, Topological selectivity of a hybrid site-specific recombination system with elements from Tn3 res/resolvase and bacteriophage P1 loxP/Cre. J Mol Biol. 289(5):1219-30 (1999) ("**Substrates with sites II and III of res close to loxP gave specific catenated or knotted products (four-noded catenane, three-noded knot) when resolvase and Cre were added together. The product topological complexity increased when the length of the spacer DNA segment between loxP and res site II was increased. Similar resolvase-induced effects on Cre recombination product topology were observed in reactions of substrates with loxP sites adjacent to full res sites.**").